--Although the improvements in the specificity and sensitivity of the EIAs have resulted in a reduction in the number of new HCV infections (Alter H.J., *Blood* 85(7):1681-1695, 1995), many investigators have indicated that the current versions still require further development. (Tobler L.H., *et al.*, *Transfusion* 34:130-134, 1994; Courouce A.M., *et al.*, *Transfusion* 34:790-795, 1994; Damen M., *et al.*, *Transfusion* 35:745-749, 1995; Feucht H.H., *et al.*, *J. Med Virol.* 48:184-190, 1995; Bar-Shany S., *et al.*, *Inetrnl. J. Epi.* 25:674-677, 1996; Dhaliwal S.K., *et al.*, *J. Med. Virol.* 48:184-190, 1996; Pawlotsky J.M., *et al.*, *J. Clin. Micro.* Jan:80-83, 1996) The impetus to improve tests for detection of anti-HCV is based upon studies demonstrating that currently available EIAs have relatively poor specificities, especially in low-prevalence populations. (Alter H.J., *Blood* 85(7):1681-1695, 1995; Feucht H.H., *et al.*, *J. Med. Virol.* 48:184-190, 1995) Additionally, even after the development of supplemental tests, such as MATRIX immunoassay (Abbott Laboratories, Abbott Park, IL), used to confirm EIA positive sera, 10% of specimens are still classified as indeterminate (reactive to a single antigen) following supplemental testing. (Pawlotsky J.M., *et al.*, *J. Clin. Micro.* Jan:80-83, 1996) These findings might be due to testing sera during the very early stage of infection before all antibodies reach detectable levels. Alternatively, reactivity to a single antigen may be due to non-specificity of the specimen.--

C1

On page 6 of the specification, please replace the description of Figure 11 with the following:

C2

--Figure 11 shows the analysis of selected expressed and purified protein by immunoblot analysis. A single anti-HCV positive human sera with high anti-NC activity by MATRIX immunoassay was used as the source of primary antibody.--

On page 6 of the specification, please replace the description of Figure 13 with the following:

C3 Figure 13 shows an endpoint titration of specimen no. BBI 304 by NC Mosaic EIA and by MATRIX immunoassay for the detection of anti-NC activity.

On page 6 of the specification, please replace the description of Figure 14 with the following:

C4 Figure 14 shows an endpoint titration of specimen no. BB1325 by NC Mosaic EIA and by MATRIX immunoassay for the detection of anti-NC activity.

On page 6 of the specification, please replace the description of Figure 15with the following:

C5 Figure 15 depicts a comparison of the NC Mosaic EIA (OD y-axis) and MATRIX immunoassay (S/C y-axis) for the detection of anti-NC activity in seroconversion no. 4812.

On page 6 of the specification, please replace the description of Figure 16 with the following:

C6 Figure 16 depicts a comparison of the NC Mosaic EIA (OD y-axis) and MATRIX immunoassay (S/C y-axis) for the detection of anti-NC activity in seroconversion no. 4813.

On page 6 of the specification, please replace the description of Figure 17 with the following:

C7 Figure 17 depicts a comparison of the NC Mosaic EIA (OD y-axis) and MATRIX immunoassay (S/C y-axis) for the detection of anti-NC activity in seroconversion no. 4814.

On page 7 of the specification, please replace the description of Figures 22A-B with the following:

C8 --Figures 22A-B shows the endpoint titration of specimen no. 1 (Figure 22A) and no. 2 (Figure 22B) by the NS4 Mosaic EIA and by MATRIX immunoassay.--

On page 7 of the specification, please replace the description of Figure 23 with the following:

C9 --Figure 23 shows a comparison of the percent positively for anti-NS3, anti-NS4, and anti-NC activity by MATRIX immunoassay versus anti-NS4 activity by NS4 Mosaic EIA using 182 anti-HCV positive sera.--

On page 7 of the specification, please replace the description of Figure 24 with the following:

C10 --Figures 24A-D demonstrate the sensitivity of the NS4 Mosaic EIA versus MATRIX immunoassay for the detection of anti-NS4 activity in seroconversion panel nos. 4811 (Figure 24A), 4812 (Figure 24B), 6214 (Figure 24C) and 4813 (Figure 24D).--

On page 7 of the specification, please replace the description of Figure 25 with the following:

C11 --Figure 25 shows the reactivity of genotype specific sera by NS4 Mosaic EIA for anti-NS4 activity.--

On page 19 of the specification, please replace the paragraph bridging pages 19 and 20 with the following paragraph:

C12 --To verify the immunoreactivity of each fragment, the GST-mosaic fusion proteins were analyzed by immunoblot using an anti-HCV positive sample having high anti-NC activity by MATRIX immunoassay. Nitrocellulose membranes containing immobilized proteins were incubated for 1 hour with anti-NC positive human sera diluted 1:200 times in washing solution (0.1M PBS, pH 7.2, containing 1% BSA, and 0.5% Tween 20). The membranes were washed three times with washing solution and then incubated for 1 hour with affinity-purified goat anti-

human immunoglobulin G conjugated to horseradish peroxidase (Biorad, Richmond, CA) diluted 1:5000 in washing solution. After washing, diaminobenzidine and hydrogen peroxide were added to develop the color reaction. As shown in Figure 11 (asterisks indicate the location of specific immunoreactivity), each of the purified proteins demonstrated immunoreactivity suggesting the accessibility of immunoreactive epitopes. The monomers were the least immunoreactive, and as the fragments increased in size they became increasingly more immunoreactive. Many of the lanes corresponding to the higher molecular weight fragments demonstrate specific reactivity to proteolytic cleavage products. Although Figure 11 shows data for 16 of the 21 proteins, the remaining 5 proteins behaved in a similar manner.--

On page 21 of the specification, please replace the paragraph commencing on line 14 and ending on line 33 with the following:

--Two serially diluted specimens, BBI 304 and BBI 325 were tested by the NC Mosaic EIA and by MATRIX immunoassay to determine relative sensitivities. The results were expressed as sample to cutoff values (S/CO) so that each test may be directly compared (Figures 13 and 14, respectively). A S/CO value greater than 1 is considered positive. Specimen BBI 325 reached an endpoint by MATRIX immunoassay at a dilution of 1:256,000. NC Mosaic EIA gave a S/CO value of 1.8 at that dilution; however, an examination of cutoff values at a 1:64,000 dilution and at a 1:128,000 dilution suggests that the S/CO value for the EIA may not be accurate and that the true endpoint by NC Mosaic EIA may be at a dilution of 1:32,000 or 4-fold less sensitive than MATRIX immunoassay. Conversely, Specimen BBI 304 gave an endpoint titer of 1:128,000 by MATRIX immunoassay, while the NC Mosaic EIA was still positive at a dilution of 1:256,000 suggesting that the EIA was 2-fold more sensitive than MATRIX immunoassay. It is not unusual for several samples to give different endpoint titers since the immunologic targets

C13

are very different. The endpoint titers obtained by these two assays on the same sera most probably is a reflection of the relative titers of antibodies to different antigenic epitopes as they are presented within each test format.

On page 21 of the specification, please replace the paragraph bridging pages 21 and 22 with the following:

--To measure clinical sensitivity several seroconversion panels (No. 4812, 4813, and 4814) were tested by the NC Mosaic EIA and by MATRIX immunoassay (Figures 15, 16, and 17, respectively). A cutoff value of 2.5 times background was used for the NC Mosaic EIA, while a S/CO value greater than 1.0 was used for MATRIX immunoassay. All three seroconversion panels detected anti-NC activity at approximately the same number of days after transfusion.--

C14

On page 22 of the specification, please replace the paragraph commencing on line 4 and ending on line 17 with the following paragraph:

--Another manner to measure clinical sensitivity is to test a panel of anti-HCV positive sera for anti-NC activity by NC Mosaic EIA and by MATRIX immunoassay. A panel of 128 specimens obtained from professional plasma donors tested positive by a commercially available EIA screening assay. Among the 128 initially reactive specimens, 109 were confirmed as positive by MATRIX immunoassay, while 12 tested as indeterminate and 7 as negative. Among the 109 confirmed anti-HCV positive specimens, 101 (92.6%) demonstrated anti-NC activity by MATRIX immunoassay and 99 (90.8%) by NC Mosaic EIA suggesting a slightly higher sensitivity for MATRIX immunoassay. Among the 12 indeterminate specimens, 6 demonstrated anti-NC activity by MATRIX immunoassay, and 3 by NC Mosaic EIA suggesting a higher

C15

C15 specificity for the NC Mosaic EIA. None of the 7 anti-HCV negatives were positive for anti-NC activity by either test. (Data not shown).--

On page 22 of the specification, please replace the paragraph commencing on line 18 and ending on line 27 with the following paragraph:

--In another study, among 78 initially reactive specimens 66 were confirmed as anti-HCV positive by MATRIX immunoassay, one specimen tested indeterminate, while 3 tested as negative. The NC Mosaic EIA gave concordant results with MATRIX immunoassay for anti-NC C16 activity for the 66 positive samples and for the one negative specimen. The indeterminate specimen tested negative for anti-NC activity by NC Mosaic EIA suggesting a higher specificity for this specimen. The remaining 8 specimens were known to have nonspecific reactivity to the NS4 antigen, but tested negative by both assays for anti-NC activity. (Data not shown).--

On page 22 of the specification, please replace the paragraph bridging pages 22 and 23 with the following paragraph:

--Finally, 23 anti-HCV sera representing genotypes 1 - 5 were tested for anti-NC activity by NC Mosaic EIA and by MATRIX immunoassay. The results indicating a 100% concordance between the two assays (data not shown) indicating that the mosaic NC protein, although C17 composed of sequences from genotypes 1 - 3, contains crossreacting epitopes that react with anti-NC positive sera obtained from individuals infected with 5 different genotypes. Collectively, these results suggest that the NC mosaic protein when used as the immunologic target in an EIA format is at least as sensitive and possibly more specific than MATRIX immunoassay for the detection of anti-NC activity.--

On page 27 of the specification, please replace the paragraph commencing on line 5 and ending on line 13 with the following paragraph:

--Anti-HCV positive sera were obtained from Boehringer Mannheim Inc. (Penzberg, Germany) and from Boston Biomedical Inc. (West Bridgewater, MA). Anti-HCV negative sera were obtained from a collection of normal human blood donors reposited at the Centers for Disease Control and Prevention (CDC, Atlanta, GA). All sera were confirmed as anti-HCV positive or negative by EIA and initially reactive specimens were confirmed and further characterized by the supplemental test MATRIX immunoassay (Abbott Laboratories, Abbott Park, IL).--

C18

On page 28 of the specification, please replace the paragraph commencing on line 8 and ending on line 24 with the following paragraph:

A statistically valid cutoff value was determined by screening 160 anti-HCV negative sera and 166 anti-HCV positive sera (anti-NS4 positive by MATRIX immunoassay) by EIA. The results showed that approximately 90% of anti-HCV negative sera gave OD values less than 0.09, while approximately 80% of anti-HCV positive sera gave OD values greater than 2.1. The mean OD value for the anti-HCV negative specimens was $0.0518 \pm 0.0273$ standard deviations (SD). The cutoff value was established as the mean of OD values for anti-HCV negative sera plus 3.5 times the SD of the mean. This cutoff value unambiguously separated the negative sera from the positive sera (Figure 21), although one negative specimen gave an OD value slightly above this cutoff. Using this cutoff value, all of the anti-HCV positive specimens tested positive by the NS4 Mosaic EIA. A two by two analysis of the data revealed a sensitivity of 100% and a specificity of 99.4% using this derived cutoff value. By raising the cutoff to the mean + 4.3 times the SD, the specificity compared to MATRIX immunoassay was 100%.

C19

On page 28 of the specification, please replace the heading beginning on line 26 and ending on line 27 with the following:

N.E.

C20 *NS4 Mosaic EIA Compared to MATRIX Immunoassay on Serially Diluted Anti-HCV Positive Sera*

On page 28 of the specification, please replace the paragraph bridging pages 28 and 29 with the following paragraph:

C21 --To examine the antigenic reactivity of the NS4 mosaic protein in detecting anti-NS4 activity, two serially diluted anti-NS4 positive sera were tested by the NS4 Mosaic EIA and by MATRIX immunoassay. The results showed that anti-NS4 antibody can be detected by the NS4 Mosaic EIA at a dilution of 1:128,000 times, while MATRIX immunoassay was positive for anti-NS4 activity at a dilution of approximately 1:4000. MATRIX immunoassay utilizes two different NS4 proteins expressed in *E. coli* and in yeast. This comparison indicated that the antigenic reactivity to the NS4 mosaic protein was 32 times more sensitive than MATRIX immunoassay for specimen no. 1 (Figure 22A) and 18 to 25 times more sensitive for specimen no. 2 (Figure 22B).--

On page 29 of the specification, please replace the heading beginning on line 4 and ending on line 5 with the following:

C22 --*NS4 Mosaic EIA Compared to MATRIX Immunoassay for the Detection of Anti-HCV*--

On page 29 of the specification, please replace the paragraph commencing on line 6 and ending on line 16 with the following paragraph:

C23 --Among 182 anti-HCV positive sera, 97.8% tested positive for anti-NS4 activity by the NS4 Mosaic EIA compared to 86.8% by MATRIX immunoassay. These results strongly suggest that the mosaic protein is a more sensitive immunologic target than either of the NS4 antigens used by MATRIX immunoassay. Antibody activity to the NS3 and nucleocapsid (NC) antigens by MATRIX immunoassay were also compared to the mosaic protein for anti-NS4 activity. This

C23 analysis showed that 98.4% of the 182 sera tested positive for anti-NS3 and 94.5% for anti-NC indicating that the NS4 Mosaic EIA is more sensitive than MATRIX immunoassay for anti-NC activity, and almost as sensitive as MATRIX immunoassay for anti-NS3 activity (Figure 23).--

On page 29 of the specification, please replace the heading beginning and ending on line 18 with the following:

C24 --*NS4 Mosaic EIA Compared to MATRIX Immunoassay for Seroconversion Panels*--

On page 29 of the specification, please replace the paragraph commencing on line 19 and ending on line 34 with the following paragraph:

--Ten seroconversion panels (BioClinical Partners, Inc.; Serologicals, Chamblee, GA) were tested by the NS4 Mosaic EIA and by MATRIX immunoassay to determine the temporal appearance of anti-NS4 activity in recently infected individuals. The results showed that the NS4 Mosaic EIA detected anti-NS4 activity approximately 15 (Figure 24) to 25 days (Figure 24) earlier than MATRIX immunoassay when a cutoff value of at least 2.5 times background was used. In some cases, the NS4 Mosaic EIA and MATRIX immunoassay gave similar results; however, MATRIX immunoassay results never demonstrated earlier detection of anti-NS4 activity than NS4 Mosaic EIA results (data not shown). These results indicate that the NS4 mosaic protein, when used as the immunologic target in an EIA, was at least as sensitive as MATRIX immunoassay for the early detection of anti-NS4 activity, and probably more sensitive if more frequent bleed dates were available for each of the ten seroconversion panels.--

C25

On page 29 of the specification, please replace the paragraph bridging pages 29 and 30 with the following paragraph:

C26 --Since the NS4 mosaic protein is composed of antigenic regions derived from several HCV subtypes and genotypes, it should detect anti-NS4 activity in the sera from patients infected